These meetings have been the most intensely concerned gatherings of researchers and potential processors in the promising preservation methods having special merit for military supplies. This country's principal authorities serve on our panels to guide us to safety and regulatory acceptance. The final effort now appears possible to effect practical conclusions of restricted but meaningful food applications of irradiation. Persistence and faith added to scientific evidence have to culminate in success especially at this location, identified with the "work ethic."

TOXICOLOGY STUDIES OF GAMMA AND ELECTRON STERILIZED CHICKEN

DRS. DONALD W. THAYER and EUGENE WIERBICKI*

ABSTRACT

A large comprehensive toxicological study of chicken sterilized by ionizing radiation was initiated by the U.S. Army in 1976 at Raltech Scientific Services in St. Louis, MO. Five diets were compared in the studies: (1) 100% basal diet, (2) 35% enzyme-inactivated chicken, stored frozen, (3) 35% thermally sterilized chicken, (4) 35% gamma-ray (5.9 Mrad) sterilized enzyme-inactivated chicken, and (5) 35% electron (5.9 Mrad) sterilized enzyme-inactivated chicken. None of the four processed chicken meats induced a teratogenic response in the mouse, hamster, rat, and rabbit, nor was there an effect on the response to known mutagens in the Ames test. Based on the breeding performance of male offspring of male mice which ate the diets, and examination of the testicular cells, there was no evidence of chromosome damage. Nor were dominant lethal effects observed in mice that had consumed the chicken diets. None of the processed meat samples induced sex-linked recessive lethal mutations in Drosophila melanogaster. However, an unexplained significant reduction occurred in the number of progeny of D. melanogaster reared on gamma irradiated chicken. Chronic toxicity, oncogenicity, and multigeneration reproductive studies were completed with beagle dogs and CD-1 mice. A study with rats was terminated after 39 weeks. No treatment related abnormalities or changes were noted in the beagle dogs, but survival of both sexes of certain subgroups of the CD-1 mice fed gamma-irradiated chicken was significantly reduced. The mouse group receiving gamma-treated chicken also had the highest incidence of tumors and lesions.

INTRODUCTION

In 1968 the FDA rescinded the approval for gamma-irradiated canned bacon citing apparent adverse effects produced in animals fed the irradiated bacon and significant deficiencies in portions of the experimental design (Spiher, 1968; Takeguchi, 1981). The FDA and the National Research Council of the National Academy of Sciences cooperated with Army scientists to develop new protocols for greatly expanded animal feeding studies of irradiated beef, ham, pork, and

*USDA-ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118

chicken. The study of irradiation sterilized chicken products was contracted to Raltech Scientific Services of St. Louis, MO in 1976. Responsibility for supervision of the contract was transferred from the U.S. Army to the Dept. of Agriculture in October 1980. The final reports for the study were accepted during 1983, and the final copies of the reports received from Raltech Scientific Services in 1984. These reports and others were submitted to the FDA for their information on March 22, 1984 (Thayer, 1984). This report summarizes the major findings of the toxicological studies of irradiation sterilized chicken products.

MATERIALS & METHODS

Chicken meat was processed for the toxicology study as indicated in Table 1. The processing technology used for the preparation of the massive amount of enzyme inactivated chicken meat and the packaging technology was described by Wierbicki (1984) and Killoran (1984). The radappertization of the chicken by electron beam or gamma-ray energy is outlined in Table 2 and complete details provided by Wierbicki (1984).

Five diets were evaluated: (1) 100% Commercial Laboratory Diet (CLD): Formulab Chow or Lab Canine Diet. (2) Frozen Control Chicken (FC): Boneless, enzyme-inactivated (heated to an internal temperature of 75-80°C) chicken was frozen and incorporated as 35% of the total diet. (3) Thermally Processed Chicken (TP): Boneless, enzyme-inactivated chicken was thermally sterilized at 115.6°C to F_o=6.0 and incorporated as 35% of the total diet. (4) Cobalt-60 Irradiated Chicken (GAM): Boneless, enzyme-inactivated, canned in vacuo chicken was sterilized with gamma radiation from ^{60}Co (5.9 Mrad at -40±10°C) and incorporated at 35% of the total diet. (5) Electron-Irradiated Chicken (ELE): Boneless, enzyme-inactivated chicken was vacuum packed in laminated foil packages and sterilized by 10 Mev electron irradiation (5.9 Mrad at -40±10°C) and incorporated at 35% of the total diet. The protocol for the complete study was published previously (Baker and Chandler, 1984).

Teratology Studies

In each case meat diets were provided ad libitum during gestation at 35% and 70% of the total diet. Twenty confirmed pregnant CD-1 mice per diet group (total 240 mice) were provided the test and control diets from day 1 through day 18 of gestation. Thirty Golden Syrian hamsters per diet group (total 360 animals) were provided the test and control diets from day 6-10 of gestation. Thirty-three Sprague-Dawley pregnant rats per diet group (total 396) were provided the test and control diets on days 1-20 of gestation. Twenty pregnant New Zealand white strain rabbits were provided the test and control diets from days 6-18 of gestation. The positive control substances were all-trans-retinoic acid for mice, hamsters, and rats, and thalidomide for rabbits. At laparotomy the number of live and dead fetuses, early and late resorptions, number of implantation sites, gross external abnormalities, and the internal development of each fetus examined according to procedures described by Wilson and Warkany (1973).

Genetic Studies

Male CD-1 mice were selected from the F_{2a} generation of the chronic toxicity oncogenicity and multigeneration reproductive study and females from the untreated breeding colony. The number (18-36) of male mice for each diet depended on the number of available F2a litters per group. Each male was mated with nine females. Females were sacrificed 2 weeks after the midpoint of the week of mating. The smallest number of females per test diet was 162 for thermally processed chicken and the average number was 223 animals per test. Laparotomies were performed, corpora lutea examined, implantation sites identified and counted, and live and dead fetuses counted.

The Canton-S type male Drosophila melanogaster were exposed to the test diets as they developed from eggs through the three larval in-

Table 1--Processing of chicken meat for animal feeding studies

297,891 pounds of enzyme-inactivated meat

Source: 3-3.5 lb carcass weight broilers

Deboning: Hand

Processing:

Formula: 18% skin, 82% lean meat

Skin contained about 40% fat
Lean meat contained about 6% fat
Processed chicken meat contained 12-13% fat

Additives: NaCl, 0.75%
Na tripolyphosphate, 0.30%

Enzyme Inactivation:

Mixing--meat, skin, and additives mixed in vacuo
Casing--cellulose
Temperature - 75-80°C (internal)
Yield--87% of formula weight
Total time-- 9-11 hours

Table 2--Radappertization of chicken processed for toxicology studies

12-D dose for *Clostridium botulinum* spores 4.3 Mrad

GAM-Group

Cobalt-60

Temperature:	-40°C ± 10°C
Dose:	4.5 to 6.8 Mrad
Dose rate:	5.1-6.7 x 10^4 rads/min
Temperature	rise in product: 20-29°C
Package:	404 x 309 cans

ELE-Group

Electron Accelerator

Energy:	9.7 to 10 Mev
Dose:	4.5-6.8 Mrad
	avg 5.9 Mrad
Temperature	rise in product: 30°C
Package:	8 oz 2.6 cm thick slice in aluminum laminated plastic pouch

stars to pupae and as adults. Fifteen adult males were treated with each chicken sample. Twenty-five offsprings from each of four broods produced by the treated males were mated. This was repeated six times yielding approximately 9000 tests for sex-linked recessive lethal mutations per diet.

The Ames et al. (1975) Salmonella-mammalian microsome mutagenicity test was conducted on each diet for the presence of mutagenic activity. The five test bacteria and chicken extract or mutagen were incubated together for 2 hours at 22°C followed by a wash, centrifugation, and resuspension. Details of technique are provided in the report (Kuzdas et al., 1980).

Twenty male mice, 97-107 days of age, were selected from the F_{1b} generation of each group of the mouse reproduction study for mating to two stock females each. Litters produced from this mating were reared to weaning and the male pups retained for further testing for heritable translocations. The fertility of these sons of treated males were evaluated (Sheu et al., 1978; Generoso et al., 1978).

The protocol for the chronic toxicity, oncogenicity, and multigeneration reproductive study with beagle dogs is presented in Fig. 1. The protocol for the chronic toxicity, oncogenicity, and multigeneration reproductive study with CD-1 albino mice is described in Fig. 2. The rat feeding study was to parallel the mouse study. Sprague-Dawley rats were chosen for the study.

RESULTS & DISCUSSION

The positive control, triethylenemelamine, did not produce observable dominant lethal effects when administered in a series of five weekly injections of 0.075 mg/kg body weight. Between-group comparisons at laparatomy of the pregnant females fed diet groups FC and TP, and the irradiated chicken-containing diet groups GAM and ELE did not produce differences in response that were both statistically and biologically significant. Thus, no evidence was observed for dominant lethal effects as a result of the consumption of any of the test diets.

None of the samples of chicken meat (FC, TP, GAM, and ELE) were mutagenic in the sex-linked recessive lethal test with Drosophila melanogaster. The positive control containing 100 ppm tris (2,3-dibromo-propyl) phosphate produced a positive response in the test. There was, however, an unexplained significant reduction in the reproduction of Drosophila reared on gamma irradiated chicken (GAM). This response was dose-related and was not overcome by the addition of vitamin supplements or by the use of a different lot of gamma irradiated chicken meat. There was also a lower dose response to the frozen control chicken meat.

The manner in which the chicken meat was processed had no effect on the response of Salmonella tester strains to known mutagens in the Ames test. The preincubation tests with TP, GAM, ELE, and FC chicken, both with and without added mutagens, did not produce a positive response in the absence of an added mutagen.

Based on the breeding performance by male offspring of treated male mice and the examination of testicular cells from these mice, the test diets did not produce heritable translocations.

The teratology studies were conducted with mice, hamsters, rats, and rabbits, and included diets containing 35% and 70% of the test meats. None of the processed chicken meats produced a teratogenic response when fed to pregnant animals.

The planned 2-year chronic toxicity, oncogenicity, and multigeneration study of Sprague-Dawley rats fed the five experimental diets was aborted at the 39th week because of a high incidence of neonatal death in all groups. Although the cause of death was attributed to a lack of lactation, the lactation problem was not itself linked to any of the

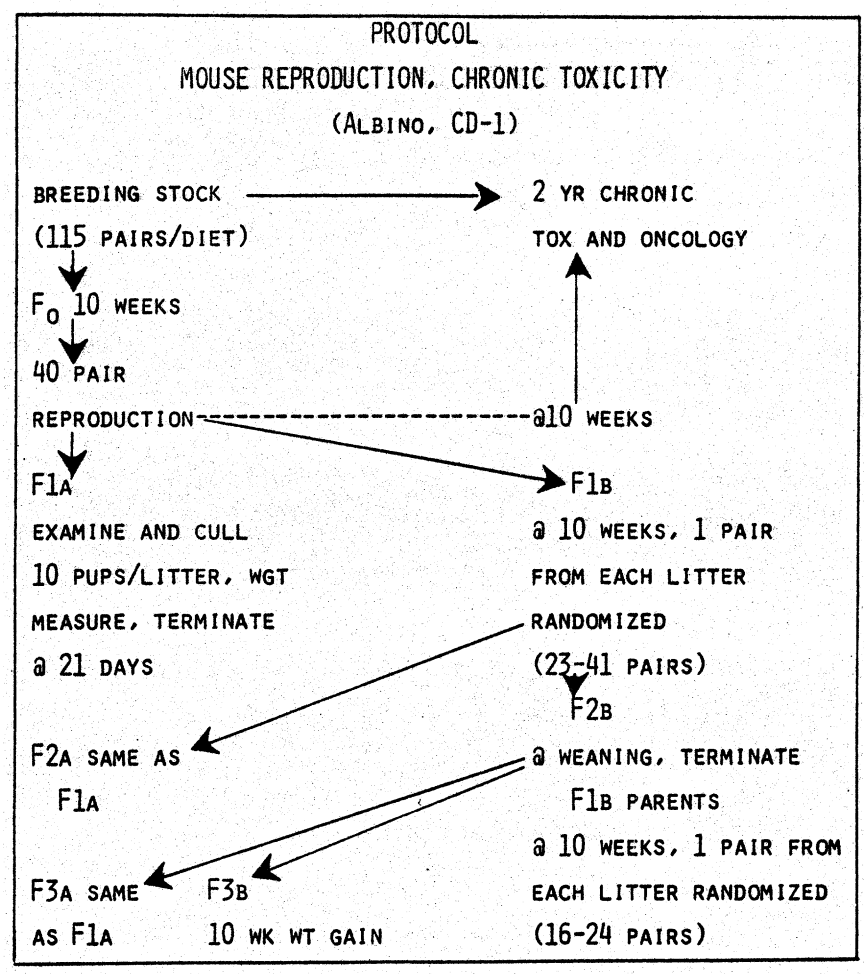

FIG. 1--PROTOCOL FOR THE MOUSE CHRONIC TOXICITY, ONCOGENICITY AND MULTIGENERATION REPRODUCTIVE STUDY.

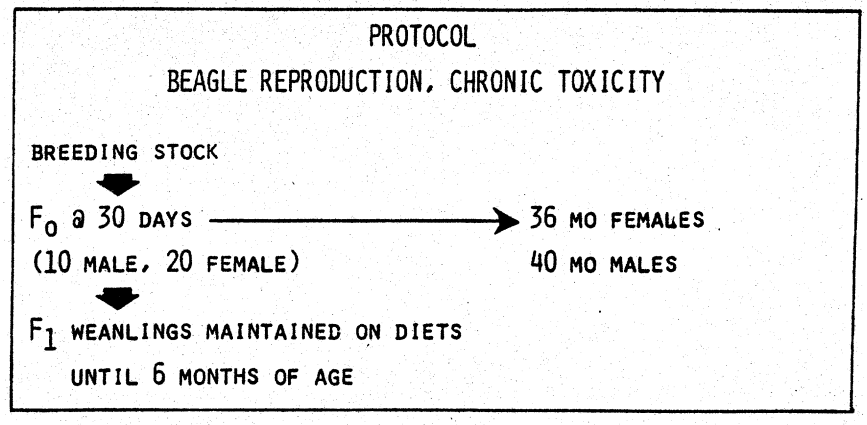

FIG. 2--PROTOCOL FOR THE DOG CHRONIC TOXICITY, ONCOGENICITY AND MULTIGENERATION REPRODUCTIVE STUDY.

chicken diets. Adverse effects from consumption of any of the five test diets were not observed on body weight, food consumption, food consumption to weight gain conversion, reproductive performance, clinical signs, behavior, ophthalmoscopy, hematological, or biochemical parameters.

Beagle dogs were exposed to the five test diets in utero. The exposure continued until death or sacrifice at 36 months postweaning for females and 40 months for males. As the F_0 females attained sexual maturity, they were bred on successive estrus periods to produce as many litters as possible before the end of the study. Offspring were selected from the F_1 generation litters at weaning for continued feeding until 6 months of age. No overt signs of toxicity attributable to diet were observed. Male F_0 dogs fed the gamma-irradiated chicken diet had significantly lower body weights through adulthood than dogs fed the frozen control diet. The investigators, however, considered the dogs in the F_0 control group to be obese. The group of F_0 females fed the gamma-irradiated diet had comparatively greater fecundity than dogs on other diets. No treatment related abnormalities or changes were observed in the dogs.

The chronic feeding study of CD-1 mice was continued for 24 months postweaning. Three generations were included in the study. No differences were observed in fertility, fecundity, stillbirth incidence, or birth-to-weaning survival in groups of mice fed gamma- (GAM) or electron- (ELE) radappertized chicken, compared to mice fed the frozen control chicken. The fertility of mice fed the thermally processed chicken was decreased. A significantly higher mortality was observed among the male F_0 mice in the upper weight quartile as compared to males from the same quartile in other groups. Mortality rates overall did not differ among groups FC, GAM, and ELE. No specific pathology was observed in the heavy GAM males. Survival of both sexes in group GAM was significantly reduced compared to controls. Group GAM had the highest incidence of several tumors, i.e., alveologenic (females), and interstitial cell testicular tumors. Nine cases of the interstitial cell testicular tumor were observed in the entire study; four each in groups GAM and ELE. The FC group had one case. The study scientists (at Raltech Scientific Services) concluded that the collective results argued against a definitive conclusion that the gamma-irradiated test material was free of toxic properties when fed to mice (Roning et al., 1984).

Other scientists, from Tracor Jitco, Incorporated, who examined the report, did not agree with the significance attributed to these findings by the Raltech Scientific Services scientists. In general, they concluded that an inappropriate statistical procedure was used which was further exaggerated by an increased rate of death in the GAM females (Seifried et al., 1983).

REFERENCES

AMES, B.N., MC CANN, J., and YAMASAKI, E. 1975. Method for detecting carcinogens and mutagens with Salmonella-mammalian microsome mutagenicity test. Mutation Research 31: 347-364.

BAKER, A.W. and CHANDLER, H.K. 1984. Animal feeding study protocol for irradiation sterilized test foods. ERRC-ARS Document No. 40. PB84-186998 National Technical Information Service, Springfield, VA.

GENEROSO, W.M., CAIN, K.J., HUFF, S.W., and GOSSLEE, D.G. 1978. Heritable Translocation Test in Mice, Chemical Mutagens- Principles and Methods for Their Detection. Vol. V, (Ed.) A. Hollander. Plenum Press.

KILLORAN, J.J. 1984. Packaging materials for use during the ionizing irradiation sterilization of prepackaged chicken products. ERRC-ARS Document No. 82. PB84-186998 National Technical Information Service, Springfield, VA.

KUZDAS, C.D., THOMSON, G., and LUSSKIN, R.M. 1980. Application of the Ames mutagenicity test for the assessment of mutagenic activity in thermal processed, frozen, electron irradiated, and gamma irradiated chicken. ERRC-ARS Document No. 72. PB84-187053 National Technical Information Service, Springfield, VA.

RONNING, D.C., BLACK, C.M., CHRISTOPHER, J.P., CUCA, G.C., DAHLGREN, R.R., ISRAELSON, E.I., MIRANTI, R.A., and MONTI, K.L. 1984. A chronic toxicity, oncogenicity, and multigeneration reproductive study using CD-1 mice to evaluate frozen, thermally sterilized, cobalt-60 irradiated, and 10 Mev electron irradiated chicken meat. ERRC-ARS Document No. 41-54. PB84-187012 National Technical Information Service, Springfield, VA.

SEIFRIED, H.E., CAMPBELL, L.A., MATON, G.L., WIJNBERG, L., HILDEBRANDT, P.K., STEDHAM, M.A., MACDONALD, W., HALPIN, V.R., REEVES, E., and FALAHEE, K.J. 1983. Irradiated chicken meat toxicology evaluation final critique. Pages 103-140 in D.W. Thayer, Summary of supporting documents for wholesomeness studies of precooked (enzyme-inactivated) chicken products in vacuum-sealed containers exposed to doses of ionizing radiation sufficient to achieve "commercial sterility." ERRC-ARS Document No. 85. PB84-186980 National Technical Information Service, Springfield, VA.

SHEU, C.M., MORELAND, F.M., OSWALD, E.J., GRAN, S., and FLAMM, W.G. 1978. Heritable translocation test on random-bred mice after prolonged triethylenemelamine treatment. Mutation Research 50: 241-250.

SPIHER, A.T., JR. 1968. Food irradiation: An FDA report. FDA Papers. Food & Drug Administration, Washington, D.C.

TAKEGUCHI, C.A. 1981. Irradiated foods, criteria for deregulation. FDA By-lines, October.

THAYER, D.W. 1984. Summary of supporting documents for wholesomeness studies of precooked (enzyme-inactivated) chicken products in vacuum-sealed containers exposed to doses of ionizing radiation sufficient to achieve "commercial sterility." ERRC-ARS Document No. 85. PB84-186980 National Technical Information Service, Springfield, VA.

WIERBICKI, E.W. 1984. Technical report. Irradiation sterilized chicken products: Technology, product quality, feasibility. ERRC-ARS Document No. 84. PB84-186998 National Technical Information Service, Springfield, VA.

WILSON, J.G., and WARKANY, J. 1973. Teratology: Principles and Techniques. The University of Chicago Press, Chicago.